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PRINCIPAL INVESTIGATOR: Elizabeth Swisher

CONTRACTING ORGANIZATION: University of Washington, Seattle, WA 98195

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Elizabeth Swisher

E-Mail: swishere@uw.edu

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University of Washington
4333 Brooklyn Ave NE
Box 359472
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14. ABSTRACT

The breast and ovarian cancer susceptibility genes *BRCA1* and *BRCA2* (*BRCA1/2*) are key components of the Fanconi anemia (FA)/homologous recombination (HR) pathway of DNA repair. Previous work had shown that cancer cells with deleterious FA/HR pathway mutations are hypersensitive to poly(ADP-ribose) polymerase (PARP) inhibitors. Importantly, however, only about half of the cancer patients with germline FA/HR pathway mutations respond to PARP inhibitors, raising the question of why a substantial fraction of HR-deficient cancers are resistant to these agents in the clinic. Based on previous work in the Swisher and Kaufmann laboratories, we proposed to test the **hypothesis** that *two different conditions must be met for ovarian cancer to be hypersensitive to platinum and PARP inhibitors: The FA/HR pathway must remain disabled and NHEJ must remain intact and functional.* Although we proposed two aims, the aim in previously banked specimens was removed before the present grant was awarded, leaving us with the following aim: **Correlate biomarkers of HR deficiency and NHEJ pathway integrity in pre-treatment biopsies with response to a PARPi in a prospective single-agent PARPi phase 2 clinical trial in sporadic ovarian carcinoma.** Over the past 12 months we have i) obtained blood and tissue specimens from the phase 2 rucaparib trial (ARIEL2, ClinicalTrials.gov identifier NCT01891344), ii) completed sequencing of 75 DNA repair genes on blood samples from ARIEL2, iii) optimized sequencing and methylation protocols for small quantities of DNA obtained from formalin fixed core biopsies iv) begun analysis on clinical outcomes of ARIEL2 (not part 1) which has completed enrollment.

15. SUBJECT TERMS

ovarian cancer, drug resistance, rucaparib, phase 2, DNA repair, homologous recombination, nonhomologous end-joining (NHEJ), poly(ADP-ribose) polymerase, BRCA1, BRCA2, PARP1

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INTRODUCTION

Poly(ADP-ribose) polymerase (PARP) is an abundant nuclear enzyme that regulates five different DNA repair pathway. Building on preclinical observations that defects in homologous recombination (HR) repair, which are found in 30-50% of ovarian cancers, sensitize cells to killing by PARP inhibitor, five separate phase 3 trials involving PARP inhibitors have opened in ovarian cancer. In a recent decision the Food and Drug Administration approved the PARP inhibitor olaparib for women with recurrent ovarian cancer and inherited mutations in the *BRCA1* and *BRCA2* genes. Part 1 of the ARIEL2 biomarker clinical trial focused on enrolling predominantly women without inherited mutations in the *BRCA1* and *BRCA2* genes in order to develop a biomarker of PARPi responsiveness for non BRCA carriers. In collaboration with Scott Kaufmann (Mayo Clinic), the present synergistic translational leverage project is assessing multiple aspects of DNA repair pathway integrity in pretreatment biopsies from a large multi-institution phase 2 study of the PARP inhibitor rucaparib. In particular, the Swisher laboratory is using massively parallel DNA sequencing to assess mutations in the HR pathway, the nonhomologous end-joining (NHEJ) pathway, PARP1 and other DNA repair genes that could impact response to PARP inhibitors.

Key words: ovarian cancer, drug resistance, rucaparib, phase 2, DNA repair, homologous recombination, nonhomologous end-joining (NHEJ), poly(ADP-ribose) polymerase, BRCA1, BRCA2, PARP1,

Overall Project Summary:

To date, we have achieved all projected milestones on time.

The submission of UW IRB approvals and related material for DOD's HRPO approval/exempt finding and MTA with Clovis Oncology was completed in year 1. The phase 2 clinical trial that is providing samples for the correlative assays in the Kaufmann and Swisher laboratories (ClinicalTrials.gov identifier NCT01891344) completed enrollment in November of 2014. 204 patients were enrolled on ARIEL2 and all pre-treatment and archival biopsies were collected by Clovis Oncology. DNA from blood was collected by Clovis Oncology and transmitted to the Swisher laboratory.

Preliminary data on response rates were so promising that rucaparib was assigned breakthrough designation by the FDA and Clovis decided to amend ARIEL2 to enroll a second cohort of patients to provide additional data for FDA approval. De-identified specimens from 188 blood sample and 226 tumor samples (113 paired pre-treatment and archival samples) have been obtained.

We have completed sequencing on blood DNA from 199 cases, which was performed blinded to tumor sequencing performed by Foundation Medicine on the trial tumor samples. Note that ARIEL2 allowed enrollment of only 15 known *BRCA* mutation carriers in order to enrich for cases without germline mutations to allow adequate development and testing of the HRD biomarker. Forty-two (21.1%) cases had germline deleterious mutations in HR genes including 15 in *BRCA1*, 7 *BRCA2,* 1 ATR, 3 *BRIP1 (*one with co-existing BRCA1 mutation*),* 1 *CHEK2*, 1 *FANCI*, 2 *FANCM*, 2 *NBN, 1 RAD51B, 4 RAD51C, 2 RAD51D, 1 SLX4, 1 CDK12*, and *1 RECQL*. Two Germline *PMS2* mutations and 1 *MSH2* mutation (DNA mismatch repair gene) were also identified, which would not be expected to impact PARP inhibitor sensitivity. Other non-HR DNA repair genes with germline mutations that were not on the Foundation Medicine panel but on BROCA-HR included *ERCC2, ERCC6* (n=2), *WRN* (n=2), *POLQ* (n=2), (Table 1). Detailed mutational information is provided in table 1.

We have optimized the protocol for sequencing the core tumor biopsies and have completed that sequencing. However, data from the tumor sequencing is still progressing through the bioinformatics pipeline and should be ready shortly. We will determine whether the germline mutations are present in the tumor and add data on somatic mutations. We have optimized our *BRCA1* and *RAD51C* methylation assays to work in FFPE tissues and are testing methylation of those genes as an alternate means of down-regulation in the ARIEL2 tissue specimens.

We are currently working with Clovis to assess clinical outcomes in the recently completed ARIEL2 part 1.

	A	B	C	D	E	F	G	H
1	**Table 1. Detailed germline mutation information for 199cases from ARIEL2 from BROCA-HR and Foundation Medicine (FM)**							
2	**sample**	**FM NGS HR and DNA repair mutation**	**FM mutation inference**	**HR Deleterious muta**	**HR Del Mutation 2**	**other DNA repair mutation**	**comments**	
3	A156AO962-002	ATR_c.7215dupA (sub-threshold MAF of 2-3%)	NA	ATR c.7215dupA				
4	A514AQ251-002	BRCA1_c.1174_1213del41	Germline	BRCA1_c.1174_1213del41				
5	A146AO355-002	BRCA1_c.124_124delA	Germline	BRCA1 c.124delA				
6	A070AO365-002	BRCA1_c.1379_1380insA	Germline	BRCA1 c.1380dupA				
7	A780AN421-002	BRCA1_c.1687C>T, p.Q563X	Germline	BRCA1 c.1681>C>T, p.Q563X				
8	A146AO356-002	BRCA1_c.2042_2043insT	Indeterminate	BRCA1 c.2043dupT				
9	A780AN396-002	BRCA1_c.2238_2238delC	Germline	BRCA1 c. 2241delC				
10	A070AO370-002	BRCA1_c.2475_2475delC	Germline	BRCA1 c.2475delC				
11	A593AN540-002	BRCA1_c.5263_5264insC	Germline	BRCA1 c.5266dupC (5385insC)				
12	A070AO373-002	BRCA1_c.5263_5264insC	Germline	BRCA1 c.5266dupC (5385insC)				
13	A067AO530-002	BRCA1_c.5263_5264insC	Germline	BRCA1 c.5266dupC (5385insC)				
14	A048AO334-002	c.470T>C	Germline	BRCA1 c.5364G>A, P.T	CHEK2 I157T			
15	A653AN222-002	BRCA1_c.66_67delAG	Germline	BRCA1 c.68_69del (185delAG)				
16	A071AO014-002	BRCA1_c.66_67delAG	Germline	BRCA1 c.68_69del (185delAG)				
17	A078AO584-002	BRCA1_c.66_67delAG	Germline	BRCA1 c.68_69del (185delAG)				
18	A781AN998-002	BRCA2_c.4449_4449delA	Germline	BRCA2 c.4449delA				
19	A593AN539-002	BRCA2_c.4552_4552delG	Germline	BRCA2 c.4552delG				
20	A613AQ992-003	BRCA2_c.5946_5946delT	Germline	BRCA2 c.5964delT (6174delT)		PRKDC c.3731insC		
21	A967AO093-003	BRCA2_c.5946_5946delT	Germline	BRCA2 c.5964delT (6174delT)				
22	A065AO361-002	BRCA2_c.5946_5946delT, BRIP1_c.1372G>T_p.E458*	Germline	BRCA2 c.5964delT (61	BRIP1 p.E458X, c.1372G.T			
23	A078AO586-002	BRCA2_c.658_659delGT	Germline	BRCA2 c.657_658del				
24	A778AO804-002	BRCA2_c.7806-2A>G_p.splice	Not determined (screen failed)	BRCA2 c.7806-2A>G, BIC IVS16-2A>G		ERCC6 p.Q98X, c.292C>T		
25	A101AO795-002	BRIP1_c.2254_2255delAA	Germline	BRIP1 c.2255delTT				
26	A609AO811-004	BRIP1_c.3693_3696delAAAG	Germline	BRIP1 c.3695del4		WRN p.R1406X		
27	A314AQ070-002	CHEK2_c.246_246delC	Germline	CHEK2 c.247delC				
28	A134AO558-002	FANCI_c.1397_1397delT	Germline	FANCI c.1397delT				
29	A948AO578-003	FANCM amplification	NA	FANCM dup entire gene				
30	A084AO587-002	FANCM c.5101C>T	Germline	FANCM p.Q1701X, c.5101C>T				
31	A452AP483-002	FANCM_c.5791C>T	Germline	FANCM p.R1931X, c.5791C>T				
32	A507AO641-003	MSH2_c.2785C>T_p.R929*	Germline			MSH2.R929X, c.2785C>T		
33	A967AO099-003	NBN c.698delTGTT	Germline	NBN c.698delTGTT			(only 1 sample available)	
34	A098AO234-002	NBN_c.654_658delAAAAC	Germline	NBN c.657_661del				
35	A660AN586-002	PMS2_c.2182_2184ACT>G_p.T728fs*7	Germline			PMS2 c.2186_2187del		
36	A444AO458-002	PMS2_c.400C>T_p.R134*	Germline			PMS2 p.R134X, c.400C>T		
37	A135AO813-002	RAD51B_c.139C>T_p.R47*	Indeterminate	RAD51B p.R47X, c.139C>T				
38	A487AO821-002	RAD51C_c.572-2A>G_p.splice site 572-2A>G	Germline	RAD51C c.572-2A>G (splice)				
39	A094AO898-002	RAD51C_c.577C>T_p.R193*	Germline	RAD51C p.R193X, c.577C.T				
40	A151AO105-002	RAD51C_c.837+1G>T_p.splice	Germline	RAD51C c.837+1G>T, splice site mutation				
41	A313AQ967-002	RAD51C_loss	NA	RAD51C del exon1-5				
42	A967AO098-005	RAD51D_c.421C>T_p.R141*	Germline	RAD51D p.R253X, c.757C>T, NM_002878			different nomenclature but same mutation	
43	A662AO812-002	RAD51D_c.434_440delGCGGGAG	Indeterminate	RAD51D c.772_778delGCCTCCC			different nomenclature but same mutation	
44	A149AO833-002			CDK12 c.1047-2A>G, splice			gene not on FM panel	

	A	B	C	D	E	F	G	H
45	A511AO415-002			RECQL p.R215X, c.643C>T			gene not on FM panel	
46	A607AO700-002			SLX4 c.1406dupC			gene not on FM panel	
47	A157AO117-002					ERCC2 1732_1741delGGGCGACACT	gene not on FM panel	
48	A474AQ289-002					ERCC6 c.88delG	gene not on FM panel	
49	A120AO929-002					ERCC6/ERCC6-PGBD3.c88delG	gene not on FM panel	
50	A134AO557-002					HELQ c.3095delA	gene not on FM panel	
51	A601AQ599-003					MRE11A.del ex8-9	gene not on FM panel	
52	A948AO558-003					POLQ c.4262delAATAGTA	gene not on FM panel	
53	A615AN001-002					POLQ c.5544dupA	gene not on FM panel	
54	A664AN497-002					WRN p.R1406X, c.4216C>T	gene not on FM panel	
55	A609AO807-003					WRN p.R1406X, c.4216C>T	gene not on FM panel	
56	A135AO808-002					XRCC4 c.24delC	gene not on FM panel	
57	A174AO732-002							
58	A655AN936-002							
59	A593AN459-002							
60	A660AN587-002							
61	A593AN542-002							
62	A780AN423-002							
63	A968AN125-002							
64	A593AN461-002							
65	A780AN284-002							
66	A655AN926-002							
67	A593AN460-002							
68	A770AN232-002							
69	A655AN928-002							
70	A615AN005-002							
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80	A067AO519-002							
81	A077AO407-002							
82	A077AO370-002							
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84	A086AO234-002							
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86	A091AO014-002							
87	A090AO471-002							
88	A079AO235-002							
89	A070AO634-002							
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92	A780AN285-002							
93	A593AN510-002							
94	A075AO253-002							
95	A151AO081-002							
96	A151AO100-002							

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97	A135AO845-002							
98	A135AO803-002							
99	A146AO354-002							
100	A075AO847-002							
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102	A091AO015-002							
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104	A134AO559-002							
105	A135AO798-002							
106	A151AO075-002							
107	A948AO577-003							
108	A610AO261-003							
109	A967AO070-003							
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111	A601AQ524-003							
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136	A200AO511-002							
137	A452AP502-002							
138	A554AO264-002							
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141	A501AO058-002							
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148	A149AO332-002							

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153	A452AP511-002							
154	A506AO899-002							
155	A108AO494-002							
156	A160AO560-002							
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158	A253AO194-002							
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192	A416AO720-001							
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194	A514AQ262-001							
195	A609AO682-001							
196	A618AR766-001							
197	A662AO548-001							
198	A679AR409-001							
199	A773AR512-001							
200	A979AS235-001							

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201	A979AS239-001							

Key research accomplishments

None to date

Conclusions

The Swisher lab is on track to complete sequencing of all ARIEL2 samples by month 30 and begin putting data together with Dr. Kaufmann and correlating the combined data with clinical outcomes.

Publications

Scott CL, Swisher EM, Kaufmann SH.

Poly (adp-ribose) polymerase inhibitors: recent advances and future development. *J Clin Oncol* 2015 April 20;33(12):1397-406. PMID:25779564

Funding from this DoD award supported the collaboration between Dr. Swisher and Dr. Kaufmann in understanding predictors of PARP inhibitor responsiveness which is the major thrust of this OCRP proposal.

Abstracts and presentations

Identification of germline and somatic alterations in homologous recombination pathway genes in high grade ovarian carcinomas and response to the PARP inhibitor rucaparib in ARIEL2, Elizabeth Swisher, Clare Scott, Kevin K. Lin, Maria Harrell, James X. Sun, Sandra Goble, Amit Oza, Robert L. Coleman, Gottfried Konecny, Anna V. Tinker, David M. O'Malley, Rebecca Kristeleit, Ling Ma, James Brenton, Katherine Bell-McGuinn, Ana Oaknin, Alexandra Leary, Elaina Mann, Heidi Giordano, Roman Yelensky, Mitch Raponi, Iain McNeish, accepted for oral presentation, AACR Ovarian Cancer Meeting, Orlando, FL, October, 2015

Results of ARIEL2: a Phase 2 trial to prospectively identify ovarian cancer patients likely to respond to rucaparib using tumor genetic analysis, **:** Iain McNeish, Amit Oza, Robert L. Coleman, Clare Scott, Gottfried Konecny, Anna Tinker, David O'Malley, James Brenton, Rebecca Kristeleit, Katherine Bell-McGuinn, Ana Oaknin, Kevin Lin, Mitch Raponi Heidi Giordano, Lara Maloney, Sandra Goble, Lindsey Rolfe Roman Yelensky, Andrew Allen, and Elizabeth Swisher, plenary presentation at ASCO, Chicago, IL, June 2015

Tumor BRCA mutation or high genomic LOH identify ovarian cancer patients likely to respond to rucaparib: interim results for ARIEL2 clinical trial, Elizabeth Swisher, MD, Amit Oza, MD, FRCPC, MBBs, Robert L. Coleman, MD, FACOG, FACS, Clare Scott, MB BS PhD, FRACP, Kevin Lin, PhD, Erin Dominy, BS, Lara Maloney, BA, Sandra Goble, MS, Roman Yelensky, PhD, and Iain McNeish, MD, PhD, MRCP, presented at Society of Gynecologic Oncology Annual Meeting as Late breaking Abstract, Chicago, IL, March, 2015

Inventions, patents and licenses

None

Reportable Outcomes

None

Other achievements

None

Appendices

1. J Clin Oncol manuscript, Swisher et al

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JOURNAL OF CLINICAL ONCOLOGY BIOLOGY OF NEOPLASIA

Poly (ADP-Ribose) Polymerase Inhibitors: Recent Advances and Future Development

Clare L. Scott, Elizabeth M. Swisher, and Scott H. Kaufmann

A B S T R A C T

Poly (ADP-ribose) polymerase (PARP) inhibitors have shown promising activity in epithelial ovarian cancers, especially relapsed platinum-sensitive high-grade serous disease. Consistent with preclinical studies, ovarian cancers and a number of other solid tumor types occurring in patients with deleterious germline mutations in *BRCA1* or *BRCA2* seem to be particularly sensitive. However, it is also becoming clear that germline *BRCA1/2* mutations are neither necessary nor sufficient for patients to derive benefit from PARP inhibitors. We provide an update on PARP inhibitor clinical development, describe recent advances in our understanding of PARP inhibitor mechanism of action, and discuss current issues in the development of these agents.

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Clare L. Scott, Walter and Eliza Hall Institute of Medical Research and Royal Melbourne Hospital, Parkville, Victoria, Australia; Elizabeth M. Swisher, University of Washington, Seattle, WA; and Scott H. Kaufmann, Mayo Clinic, Rochester, MN.

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C.L.S., E.M.S., and S.H.K. contributed equally to this work.

Terms in blue are defined in the glossary, found at the end of this article and online at www.jco.org.

Authors' disclosures of potential conflicts of interest are found in the article online at www.jco.org. Author contributions are found at the end of this article.

Corresponding author: Scott H. Kaufmann, MD, PhD, Division of Oncology Research, Gonda 19-212, Mayo Clinic, 200 First St SW, Rochester, MN 55905; e-mail: kaufmann.scott@mayo.edu.

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POLY (ADP-RIBOSE) POLYMERASE INHIBITORS IN THE CLINIC

Since last reviewed in *Journal of Clinical Oncology*,[1] poly (ADP-ribose) polymerase (PARP) inhibitors have demonstrated efficacy in a number of settings, including platinum-sensitive epithelial ovarian cancer (OC)[2,3] and breast cancer (BC) with mutation in *BRCA1* or *BRCA2*.[4]

OC

PARP inhibitors have been studied most extensively in high-grade serous OC, with efficacy noted particularly in platinum-sensitive high-grade serous OC. A pivotal phase II study demonstrated that olaparib induces responses in *BRCA1/2* mutation carriers with progressive high-grade OC, with efficacy greater in, but not restricted to, platinum-sensitive OC.[5] A subsequent study comparing olaparib maintenance therapy versus placebo after response of relapsed high-grade serous OC to platinum-based therapy demonstrated progression-free survival (PFS) of 8.4 months with olaparib versus 4.8 months without (hazard ratio, 0.35; $P < .001$).[6] A preplanned subset analysis showed greatest benefit in OC with *BRCA1/2* mutations (either germline or somatic), with PFS extended from 4.3 to 11.2 months (hazard ratio, 0.18; $P < .001$).[7] These data and additional results led to approval of olaparib by the European Commission as maintenance therapy for platinum-responsive advanced OC and by the US Food and Drug Administration as fourth-line monotherapy, with both approvals limited to the subset of cases with *BRCA1/2* mutations.

Importantly, women whose OC lacked *BRCA1/2* mutations also derived benefit in the randomized olaparib maintenance trial (hazard ratio, 0.53; 95% CI, 0.33 to 0.84; $P < .001$),[7] suggesting a sensitive non–*BRCA1/2*-mutation subgroup, as predicted from preclinical studies.[8] Excitingly, a large subset of patients derived long-term benefit from olaparib, with approximately 40% and approximately 20% of women with *BRCA1/2*-mutant or *BRCA1/2*–wild type high-grade serous OC, respectively, not requiring a different therapy within 3 years after random assignment, compared with only approximately 10% and approximately 1% of those receiving placebo.[9] Olaparib also prolonged time to second subsequent therapy in both *BRCA1/2*-mutated OC (hazard ratio, 0.44; $P < .001$) and non–*BRCA1/2*-mutated OC (hazard ratio, 0.64; $P < .034$), suggesting that PARP inhibitor treatment did not make OC less responsive to platinum or other therapies, a conclusion supported by additional studies.[10] Olaparib in combination with carboplatin[11] or cediranib[12] has also shown efficacy against OC in phase I and II studies. Notably, however, hematologic toxicity prevented continuous dosing of olaparib when combined with typical carboplatin doses (area under curve of 5 every 3 weeks).[11]

A number of additional PARP inhibitors, including veliparib, rucaparib, niraparib, and BMN-673, have also shown efficacy in high-grade serous OC.[13] On the basis of the encouraging results of the phase II olaparib maintenance trial,[6,7] phase III trials

Table 1. Open and Soon-to-Open Phase III Trials of PARP Inhibitors in Ovarian Cancer

Drug	Sponsor	ClinicalTrials.gov Identifier	Trial	First Line or Relapsed	Ovarian Cancer Population*	*BRCA1/2* WT Allowed?	Platinum-Resistant Patients Allowed?
Olaparib	AstraZeneca	NCT01844986	SOLO1; GOG3004	First line	FIGO stage IIIC or IV; high-grade serous/endometrioid; deleterious *BRCA1/2* mutation†; CR or PR to initial platinum	No	No
Veliparib	Abbvie		GOG3005	First line	High-grade serous/endometrioid; genomic testing at enrollment	Yes	NA
Olaparib	AstraZeneca	NCT01874363	SOLO2; ENGOT-OV21	Relapsed	High-grade serous/endometrioid; deleterious *BRCA1/2* mutation†; sensitive to penultimate platinum regimen; CR or PR to current platinum	No	No
Rucaparib	Clovis	NCT01968213	ARIEL3	Relapsed	High-grade serous/endometrioid; sensitive to penultimate platinum regimen; CR or PR to current platinum	Yes	No
Niraparib	Tesaro	NCT01847274	ENGOT-OV16; NOVA; US Oncology; others	Relapsed	Deleterious *BRCA1/2* mutation or high-grade serous with CR or PR to current platinum	Yes	No

Abbreviations: ARIEL3, Assessment of Rucaparib in Ovarian Cancer Phase 3 Trial; CR, complete response; ENGOT-OV, European Network for Gynaecological Oncological Trial Groups-Ovarian Cancer; FIGO, International Federation of Gynecology and Obstetrics; GOG, Gynecologic Oncology Group; NA, not applicable; NOVA, Niraparib in Ovarian Cancer; PARP, poly (ADP-ribose) polymerase; SOLO, Studies of Olaparib in Ovarian Cancer; WT, wild type.
*Ovarian, fallopian tube, and peritoneal cancers.
†Deleterious *BRCA1/2* mutation includes germline or somatic.

with the same design are ongoing in OC (Table 1). Each of these is also attempting to improve identification of responsive patients through analysis of biospecimens (eg, examining biomarkers of homologous recombination [HR] deficiency [HRD]).[14]

BC

Overall, PARP inihibitors have been less efficacious in BC than in high-grade serous OC,[13] perhaps reflecting the biologic heterogeneity[15,16] and low *BRCA1/2* somatic mutation rate[17] in triple-negative BC. Responses were observed in 11 (41%) of 27 patients in an initial phase II trial of olaparib in *BRCA1/2*-mutated BC.[4] In contrast, there were no responses in 23 patients with triple-negative BC regardless of *BRCA1/2* mutation status. Other PARP inhibitors, including the potent agent BMN-673,[18] have induced responses in small studies, and phase III trials are ongoing in *BRCA1/2*-mutated BC and triple-negative BC (Table 2).

Other Solid Tumor Types

Additional solid tumors contain subsets that are likely to have HRD and potentially be PARP inhibitor responsive.[19] Five percent of

Table 2. Phase III Trials of PARP Inhibitors in Other Solid Tumors

Sponsor	ClinicalTrials.gov Identifier	Trial	Treatment	Cancer Population	Biomarker
Abbvie	NCT02032277	Brightness	standard NAC plus carboplatin/veliparib or standard NAC plus carboplatin/placebo	Early-stage triple-negative breast cancer	None
AstraZeneca	NCT02032823	OlympiA	Maintenance olaparib or placebo	High-risk early-stage *HER2*-nonamplified breast cancer after adjuvant chemotherapy	*BRCA1/2* mutation
AstraZeneca	NCT02000622	OlympiaD	Olaparib or physician's choice	Advanced breast cancer	*BRCA1/2* mutation
Abbvie	NCT02163694		Paclitaxel/carboplatin plus veliparib or paxlitaxel/carboplatin plus placebo	Advanced *HER2*-nonamplified breast cancer	*BRCA1/2* mutation
Tesaro	NCT01905592	BRAVO	Niraparib or physician's choice	Second-line or beyond *HER2*-nonamplified breast cancer	*BRCA1/2* mutation
AstraZeneca	NCT02184195	POLO	Maintenance olaparib or placebo	Pancreatic cancer after first-line platinum-based chemotherapy	*BRCA1/2* mutation
AstraZeneca	NCT01924533		Paclitaxel/olaparib or paclitaxel/placebo followed by maintenance olaparib or placebo	Progressive gastric cancer, second line	None
Abbvie	NCT02106546		Paclitaxel/carboplatin plus veliparib paclitaxel/carboplatin plus placebo	First-line advanced squamous non–small-cell lung cancer	None
Abbvie	NCT02152982		Temozolamide plus veliparib or temozolomide plus placebo	First-line glioblastoma	MGMT promoter hypermethylation

Abbreviations: BRAVO, Niraparib Versus Physician's Choice in Her2 Negative, Germline BRCA Mutation-Positive Breast Cancer; MGMT, O6-methylguanine-DNA methyltransferase; NAC, neoadjuvant chemotherapy; PARP, poly (ADP-ribose) polymerase; POLO, Olaparib in gBRCA Mutated Pancreatic Cancer Whose Disease Has Not Progressed on First Line Platinum-Based Chemotherapy.

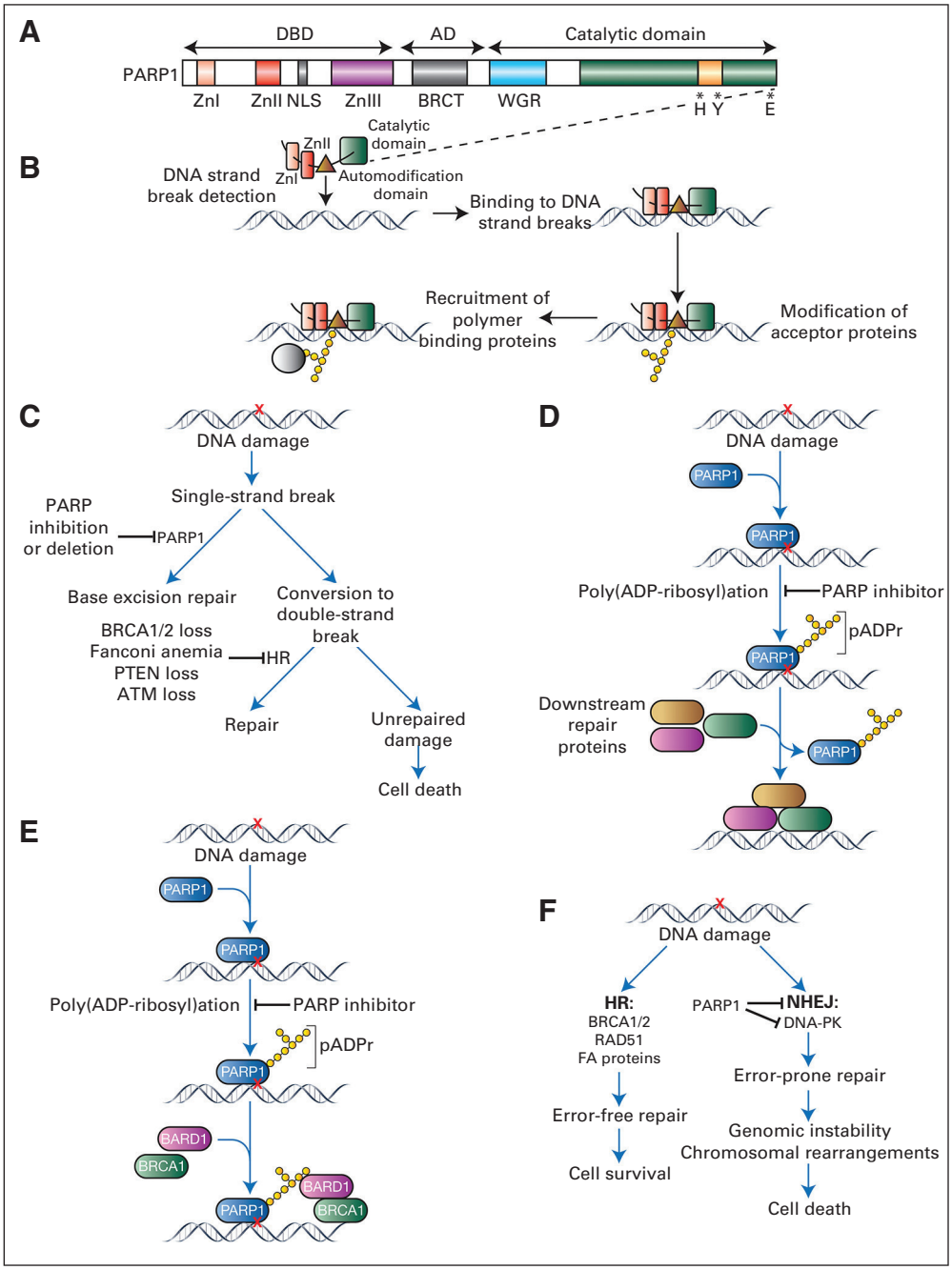

Fig 1. Summary of poly (ADP-ribose) [pADPr] polymerase 1 (PARP1) structure, function, and proposed contribution to synthetic lethality. (A) Schematic of PARP1 structure. (B) On binding to damaged DNA, PARP1 undergoes conformation change that increases its catalytic activity, leading to cleavage of NAD^+ and addition of ADP-ribose units to various proteins, including its own automodification domain. Resulting pADPr polymers (depicted as chains of yellow circles) alter function of proteins that are modified (eg, by decreasing affinity of PARP1 for damaged DNA)[29] and also recruit additional proteins that bind to polymer noncovalently.[30,31] (C-F) Models proposed to explain observed synthetic lethality between homologous recombination (HR) deficiency and PARP inhibition. These models emphasize (C) role of PARP1 in base excision repair, (D) recruitment of DNA repair proteins, (E) recruitment of BARD1-BRCA1 complex, and (F) suppression of nonhomologous end joining (NHEJ). AD, automodification domain; BRCT, *BRCA1* C-terminal domain; DBD, DNA binding domain; FA, Fanconi anemia; NLS, nuclear localization signal; PK, protein kinase; WGR, tyrptophan-glycine-arginine-rich domain; Zn, zinc finger.

cutaneous melanomas and gastric cancers, 5%-19% of familial pancreatic cancers, and 1% of prostate cancers harbor germline *BRCA1/2* mutations, with encouraging reports of responses to olaparib in *BRCA1/2*-mutant pancreatic and prostate cancers.[20] Clinical trials of single-agent PARP inhibitor treatment are ongoing in additional tumor types, with responses reported in melanoma, PTEN-deficient endometrial cancer, and colorectal carcinoma.[19]

Unanswered Questions

At present, it remains unclear how to best identify patients who will respond to PARP inhibitors. Although tumor phenotypes can provide rough predictions, as evidenced by responses of sporadic triple-negative BC[13,21] and high-grade serous OC to PARP inihbitor monotherapy,[21] the response rates are lower than for *BRCA1/2*-mutant BC or OC.[13] Accordingly, it seems that optimal clinical development might be advanced by improved understanding of both the mechanism of action of PARP inhibitors and mechanisms of resistance.

PRIMER ON PARP BIOLOGY

Since the initial description of poly (ADP-ribose) [pADPr] synthesis in the 1960s,[22,23] PARP biology has been extensively studied.[24-28] PARP1 (Fig 1A) is the founding member of a family of enzymes[24-26] that exhibit homology in their active sites, where the dinucleotide

NAD^+ binds and is cleaved during mono- or poly (ADP-ribosyl)ation of protein substrates.[26,32,33] Although 17 PARP family members have been identified in mammalian cells,[26,34] only six synthesize pADPr,[27,34] and only three (PARP1, PARP2, and PARP3) play identified roles in DNA repair.[35,36]

PARP1 is the best understood of these enzymes (Fig 1B). In cells with certain types of DNA damage, particularly nicks and double-strand breaks (DSBs),[37] PARP1 binds to damaged DNA and undergoes a conformational change that realigns critical residues in the enzyme active site,[38-40] producing an up to 500-fold increase in activity.[39,41,42] Once activated, PARP1 synthesizes pADPr chains covalently bound to a variety of chromatin proteins, although PARP1 itself is the acceptor for most of the polymer.[39,43] The resulting pADPr chains not only alter the functions of the covalently modified proteins[29,43-45] but also noncovalently bind a wide variety of additional nuclear proteins.[30,31,39,46-48]

Like other post-translational modifications, pADPr is highly dynamic. After DNA damage, polymers consisting of scores or hundreds of subunits are detectable within seconds,[41,42,49,50] resulting in rapid recruitment of additional DNA repair proteins.[49,50] Once formed, pADPr is also rapidly degraded by pADPr glycohydrolase, assuring that pADPr levels reflect persistent damage, and the response is extinguished as repair ensures.[51-53]

Through its synthesis of pADPr, PARP1 contributes to a number of DNA repair pathways.[27,28] In its most extensively studied role, PARP1 is essential for base excision repair (BER),[54-56] a process that removes a single damaged base and restores DNA integrity.[28,57] In addition, PARP1 binds to DSBs and recruits the proteins MRE11 and NBS1[49] to initiate HR,[58-60] a high-fidelity repair process that allows one copy of a gene to serve as a template for restoration of a second copy of the same gene.[28,61,62] PARP1 also poly (ADP-ribosyl)ates *BRCA1*, further contributing to and fine-tuning HR-mediated DSB repair in HR-competent cells.[63] Moreover, PARP1 prevents binding of the Ku proteins to free DNA ends,[64] thereby preventing activation of the competing but error-prone nonhomologous end-joining (NHEJ) DSB repair pathway. In addition, PARP1 is essential for microhomology mediated (alternative end-joining) repair,[65,66] a third DSB repair pathway.

PARP1 also contributes to additional cellular processes. It helps restart replication forks that stall because of nucleotide depletion or collisions with bulky lesions,[67-70] modulates gene transcription,[71] regulates chromatin structure,[71-73] alters cytoplasmic microRNA processing and action,[74] and affects energy metabolism.[27,75,76] Despite its involvement in all of these processes, however, PARP1 is not essential. *Parp1* knockout mice develop normally[77] and do not exhibit any phenotype until they encounter genotoxic stress.[54] These observations prompted the initial development of PARP inhibitors as agents to enhance targeted DNA damage.[28,78,79]

PARP2 and PARP3 also contribute to DNA repair.[27,36] PARP2 cooperates with PARP1 in synthesizing pADPr after DNA damage.[80,81] PARP3 suppresses error-prone NHEJ[82] while simultaneously partnering with PARP1 to enhance DSB repair.[83] The observation that the PARP inhibitors undergoing clinical testing interact strongly with the active sites of PARP2 and PARP3 in addition to PARP1[84] raises the possibility that effects of PARP inhibitors reflect inhibition of all three family members.

HOW PARP BIOLOGY CONTRIBUTES TO SYNTHETIC LETHALITY

Current development of PARP inhibitors as anticancer agents is motivated by the hypersensitivity of HR-deficient cells to PARP inhibition[85,86] and the ability of PARP inhibitors to sensitize cells to certain types of DNA damage.[27,28] There is emerging evidence that these two effects might reflect different aspects of PARP biology.

The observation that PARP inhibitors selectively kill *BRCA1/2*-deficient cells in preclinical models[85,86] was rapidly followed by the demonstration that additional changes leading to HRD also confer PARP inhibitor hypersensitivity.[8,87,88] At least four different aspects of PARP1 biology have been invoked to explain this so-called synthetic lethality, although each model also has limitations.

Inhibition of BER

Because PARP1 is essential for BER,[36,89] initial explanations suggested that DNA single-strand breaks (SSBs), which arise during normal cellular activity and are ordinarily repaired by BER, persist during PARP inhibitor treatment and are converted to DSBs, which are repaired by HR in HR-proficient cells but remain unrepaired in HRD cells (Fig 1C).[90,91] The inability to detect SSB accumulation during PARP inhibitor treatment,[92] however, casts doubt on this model. Moreover, knockdown of PARP1 kills HRD cells,[85,86,93] whereas knockdown of XRCC1, the protein immediately downstream of PARP1 in BER, does not,[93] suggesting that loss of PARP1 activity is critical for killing of HRD cells, but loss of BER is not.

Trapping of PARP1 on Damaged DNA

When DNA damage activates PARP1,[40,41,94] the resulting pADPr recruits additional repair proteins[30,46,47,55] and simultaneously diminishes the affinity of PARP1 for DNA,[29] allowing its dissociation so other repair proteins can bind. Conversely, PARP1 that cannot synthesize polymer remains bound to damaged DNA and inhibits DNA repair under cell-free conditions (Fig 1D).[29] Moreover, overexpression of the isolated PARP1 DNA binding domain, which also recognizes damaged DNA but cannot synthesize pADPr, potentiates certain types of DNA damage.[95,96] PARP1 that is inactivated by a PARP inhibitor would likewise be expected to bind to damaged DNA and inhibit repair. This trapping mechanism has been implicated in the synergy between PARP inhibitors and certain DNA damaging agents, including temozolomide[97,98] and topotecan.[99] Extrapolating from these observations, it has been suggested that cytotoxicity of PARP inhibitors in HRD cells might result from trapping of PARP1 at sites of endogenous damage,[100] although this mechanism fails to explain the observation that PARP1 knockdown also selectively kills BRCA1/2-deficient cells.[85,86,93]

Defective BRCA1 Recruitment

BRCA1 recruitment to damaged DNA involves two steps[50]: first, an interaction between pADPr at the damage site and the pADPr binding protein BARD1, which brings along its binding partner BRCA1, and second, an interaction of BRCA1 with γ-H2AX, a modified histone formed in response to DNA damage.[101] If *BRCA1* mutation impairs the BRCA1/γ-H2AX interaction, recruitment of the BARD1-BRCA1 complex to pADPr becomes critical for DNA repair (Fig 1E). The ability of PARP inhibitors to diminish recruitment of the BARD1-BRCA1 complex to damaged DNA, thereby

impairing DSB repair, provides an explanation for the PARP inhibitor hypersensitivity of cells with certain *BRCA1* mutations,[50] but it is unclear whether this explains PARP inhibitor hypersensitivity of cells with other HR defects.

NHEJ Activation

A fourth explanation for PARP inhibitor–induced killing focuses on the role of PARP1 in suppressing the error-prone NHEJ repair pathway (Fig 1F).[93,102] Several proteins in this pathway,[103] including Ku70, Ku80, and DNA-PKcs, are pADPr binding proteins.[30,46,47] The interactions of Ku70 and Ku80 with pADPr suppress NHEJ.[64,104,105] Conversely, PARP inhibitors de-repress NHEJ, which then becomes active in HR-deficient cells.[93] Importantly, chromosomal rearrangements and mutations, felt to be hallmarks of error-prone NHEJ,[86] are induced by PARP inhibitors and diminished by simultaneous addition of DNA-PK inhibitors to HR-deficient cells.[93] Moreover, PARP inhibitor cytotoxicity in HR-deficient cells is diminished by manipulations that inhibit NHEJ,[93,106,107] suggesting that activation of error-prone NHEJ contributes to PARPi/HRD synthetic lethality (Fig 1F). Conversely, PARP inhibitor sensitivity of HR-deficient cells is enhanced by changes that inhibit alternative end joining,[108] another DSB repair pathway that functions in parallel with HR and NHEJ. It is unclear, however, what activates the NHEJ pathway in PARP inhibitor–treated cells or how cells survive when HR and NHEJ are both disabled.

Potential Implications for Patient Selection

These models of PARP inhibitor–induced killing make different predictions regarding PARP inhibitor sensitivity and resistance.[102] The PARP trapping model (Fig 1D), for example, predicts that cancers with higher PARP1 expression will be more sensitive to PARP inhibitors (because of increased PARP1 trapping on damaged DNA), whereas the other models predict that cancers with lower PARP1 expression will be more sensitive. Furthermore, the NHEJ model (Fig 1F) predicts that changes affecting the rate of NHEJ will have an impact on PARP inhibitor sensitivity, in agreement with the observation that loss of 53BP1 (protein that facilitates NHEJ) or the NHEJ protein Ku80, DNA-PKcs, or Artemis diminishes PARP inhibitor sensitivity,[93,106,107,109-111] whereas loss of POLQ, the DNA polymerase in the alternative end-joining pathway, enhances PARP inhibitor sensitivity.[108] Accordingly, sorting out which of these models accounts for responses in the clinical setting might help identify patients more likely to respond to PARP inhibitors.

WHICH PATIENTS ARE MOST LIKELY TO RESPOND, AND HOW CAN WE BEST IDENTIFY THEM?

In the absence of more refined understanding of PARP inhibitor action, *BRCA1/2* mutation status has been the most extensively studied predictor of PARP inhibitor sensitivity to date. When PARP inhibitors are administered as single agents in the relapsed setting, *BRCA1/2*-mutated OC has a 30% to 45% objective response rate.[5,112,113] A higher response rate is observed in platinum-sensitive *BRCA1/2*-mutant high-grade serous OC than in platinum-resistant or -refractory groups,[112] but responses in cases of platinum-resistant disease[114] suggest that PARP inhibitors could also be useful in subsets of patients with resistant or refractory disease. Responses to PARP inhibitor therapy in other solid tumors that occur in families with germline *BRCA1/2* mutations, including pancreatic cancer, melanoma, and prostate cancer, have also been reported.[20]

In contrast, not all patients with deleterious *BRCA1* or *BRCA2* mutations at diagnosis respond to PARP inhibitors. In cell lines, secondary somatic mutations in *BRCA1*- or *BRCA2*-mutant cancer cells can restore protein expression, reconstitute HR, and confer resistance to PARP inhibitors and platinum.[115-117] Secondary mutations that restore BRCA1 and BRCA2 also predict platinum and PARP inhibitor resistance in the clinical setting.[118,119] It seems that approximately 45% of recurrent platinum-resistant *BRCA1/2*-mutated OCs have secondary somatic mutations.[118] Interestingly, clinical cancer specimens most commonly sustain secondary somatic mutations that revert the mutant allele to wild-type sequence, making secondary mutations highly predictive of response but technically difficult to identify.[118]

In addition to reversion mutations, HR can be restored in other ways. Some mutant *BRCA1* alleles encode proteins that are potentially functional but degraded rapidly (so-called hypomorphic alleles). Stabilization of these mutant proteins (eg, by elevated expression of heat shock protein 90) can restore HR and confer PARP inhibitor resistance without any secondary *BRCA1* mutation.[120] Likewise, decreased expression of 53BP1, which ordinarily channels DSB repair to NHEJ, restores HR and confers PARP inhibitor resistance in *BRCA1*-mutant cells despite the continued absence of BRCA1 protein.[109,110,121] The extent to which these mechanisms contribute to PARP inhibitor resistance in clinical OC remains to be fully defined.

Despite the current focus on *BRCA1/2* mutation carriers with OC, responses are not limited to this group. OCs with somatic *BRCA1/2* mutations seem to be as likely to benefit from PARP inhibitor maintenance therapy as those with inherited mutations,[7] although the number of treated patients with somatic mutations is small. Moreover, germline or somatic mutations in other genes critical to HR correlate with platinum sensitivity in OC and might also predict PARP inhibitor response.[122] Intriguing efficacy has been reported for olaparib in PTEN-deficient endometrial cancer[123] and in combination with paclitaxel in gastric cancer with *ATM* deficiency.[124] Studies including *PALB2*-mutated OC and pancreatic cancer are also under way.

In addition to mutations, other processes, including epigenetic alterations and changes in expression of microRNAs or transcription factors, could in principle impair HR and confer PARP inhibitor sensitivity. *BRCA1* promoter hypermethylation, which downregulates BRCA1 expression, occurs in 10% to 15% of OCs and has been proposed as a mechanism of HRD.[125-127] However, data from The Cancer Genome Atlas and others fail to correlate *BRCA1* hypermethylation with increased platinum sensitivity or improved survival,[128] suggesting that epigenetic BRCA1 downregulation may have a less profound impact on HR and PARP inhibitor sensitivity than inactivating *BRCA1* mutations. In short, improved understanding of PARP biology and HRD is providing important new clues for predicting PARP inhibitor responders versus nonresponders.

PARP INHIBITOR–CONTAINING COMBINATION THERAPY

Improved understanding of PARP biology is also contributing insights into the design of PARP inhibitor–containing combination

therapy. PARP inhibitors have been combined with standard chemotherapy, such as platinum in OC and BC[13] or temozolomide in melanoma, BC, glioblastoma, and acute leukemia, as well as with signal transduction inhibitors (eg, gefitinib in *EGFR*-mutant non–small-cell lung cancer).[13,19] Mechanisms underlying these combinations fall into two broad categories: first, induction of HRD and PARP inhibitor hypersensitivity in cells that initially contain an intact Fanconi anemia (FA)/HR pathway, or second, enhancement of DNA damage through interference with one of the roles of PARP1.

Previous studies have demonstrated that HRD can be induced by a variety of treatments, including epidermal growth factor receptor inhibitors[129] or cyclin-dependent kinase inhibitors,[130] which promote BRCA1 trafficking from the nucleus to the cytoplasm; phosphatidylinositol 3-kinase inhibitors, which downregulate Rad51[131] or BRCA1/2[132]; ATR inhibitors, which diminish replication stress–induced activation of cell-cycle checkpoints and repair[133]; or even PARP inhibitors themselves.[134] Whether pharmacologic induction of HRD will sensitize clinical cancers to PARP inhibitors as effectively as inactivating mutations in FA/HR pathway genes remains to be determined.

PARP inhibitors also sensitize cells to certain DNA-damaging agents.[27,28,78,79] Different modes of PARP inhibitor action depicted in Figure 1 explain these effects. For example, PARP inhibitors acting as inhibitors of BER (Fig 1C) sensitize cancer cells to the nucleoside analog floxuridine.[135,136] In contrast, sensitization to temozolomide and other methylating agents reflects the PARP trapping mechanism (Fig 1D). Not only do PARP inhibitors increase the amount of PARP1 and PARP2 bound to methylated DNA,[98,100] but diminished PARP1 protein protects cells from methylating agents,[97,137] as predicted by this mechanism. Importantly, complete PARP1 inhibition might not be required to sensitize cells through this mechanism, because trapping of only a small amount of PARP1 on the DNA should impede repair of some of the lesions and enhance cytotoxicity. This might explain the severe hematologic toxicity observed when PARP inhibitors are combined with temozolomide[138] or topoisomerase I poisons,[139] where a similar mechanism of sensitization has been reported.[99] Whether this trapping mechanism can be harnessed to selectively increase the toxicity of DNA damage in cancer cells as compared with normal tissues in the clinical setting remains to be established.

PREVIOUS BARRIERS TO CLINICAL IMPLEMENTATION

Despite the promising clinical results observed thus far, there have been a number of barriers to clinical development of PARP inhibitors, including confusion about what constitutes a bona fide PARP inhibitor as well as problems with predictive biomarkers, pharmacodynamic end points, and ideal trial design.

Implications of Accurate Mechanism of Action

PARP inhibitor development was delayed by inaccurate classification of earlier compounds. In particular, iniparib was classified as a PARP inhibitor based on its inhibition of purified PARP1.[140] When iniparib failed to enhance the efficacy of the gemcitabine/oxaliplatin doublet in triple-negative BC,[141] the entire class of PARP inhibitors was considered by many to have failed.[142] It turned out, however, that iniparib does not inhibit PARP in intact cells.[143,144] Until this was realized, the inaccurate classification of iniparib as a PARP inhibitor slowed pivotal testing of bone fide PARP inhibitors.

Identification of Predictive Biomarkers

At the present time, *BRCA1/2* loss-of-function mutations, either germline or somatic, have been the most extensively studied biomarkers of PARP inhibitor response. However, restricting PARP inhibitor development to *BRCA1/2*-mutated cancers would exclude additional cancers that may benefit. Because not all of the genes that affect DNA repair are currently known, a functional test of DNA repair capability that could be applied in the clinical setting would accelerate the identification of cancers appropriate for PARP inhibitor therapy. Initially, static tests such as immunohistochemistry or immunofluorescence for RAD51 pathway components, including RAD51 itself, were suggested as a way to determine whether DNA repair was occurring. However, antibodies to RAD51 have not proven sufficiently specific, sensitive, or reliable for clinical application.

At present, there is substantial interest in assays of genomic scarring (ie, subchromosomal amplifications and deletions thought to reflect HRD).[128,145-149] Preliminary data from both patient-derived xenografts and the ARIEL2 (Assessment of Rucaparib in Ovarian Cancer Phase 2 Trial) trial suggest that an assay using loss of heterozygosity to identify genomic scarring may be useful to predict PARP inhibitor response in OC without *BRCA1/2* mutations.[150,151] In contrast, it is important to emphasize that genomic scarring will not disappear when HR is restored by these secondary mutations, suggesting that assays of genomic scarring might need to be supplemented with assays for resistance mechanisms.[149]

Limitations of Pharmacodynamic Assays

Most early-phase PARP inhibitor trials have included measurement of pADPr to assess PARP1 inhibition. Because PARP activity can increase up to 500-fold after DNA damage,[39,41,42] it is important that 50% or even 90% PARP inhibition not be viewed as satisfactory suppression of pADPr synthesis. In early reports of failed efficacy, for example, the dose of veliparib guided by pADPr assays was 20 to 60 mg per day, which is much less than the 200 to 400 mg twice daily being delivered in veliparib trials now showing efficacy.

Limitations of Combination Trial Design

Most existing combination trials have started with the premise of adding PARP inhibitors to standard-dose chemotherapy. This has often led to administration of low doses of PARP inhibitors, which is concerning given evidence suggesting a dose-response relationship for PARP inhibitors. The alternative of using a low-dose chemotherapeutic regimen such as oral metronomic cyclophosphamide has been explored, but a standard dose of cyclophosphamide (50 mg daily) was again used, resulting in a relatively low veliparib dose (60 mg twice daily) at the maximum-tolerated dose.[152] An alternative approach of combining a near-maximal PARP inhibitor dose with lower, intermittent doses of a DNA-damaging agent such as oral cyclophosphamide should be considered.

PERSPECTIVE ON FUTURE DEVELOPMENT

With the previous considerations in mind, we offer suggestions that we hope will advance the development of PARP inhibitors.

How Can We Most Efficiently Identify Patients Who Will Benefit From PARP Inhibitors?

Patients are currently considered for PARP inhibitor trials if they have a particular tumor type (eg, high-grade serous OC or triple-negative BC) or their cancer could belong to a relevant molecular subtype (eg, *BRCA1/2*-mutated breast, ovarian, pancreatic, or prostate cancer). Given the known relationship between *BRCA1/2* mutations and PARP inhibitor responsiveness, we suggest that all PARP inhibitor trials enrolling these patients should report *BRCA1/2* mutation status for all participants (both germline and somatic), analogous to trials of any other therapy with a known molecular target.

The current focus on *BRCA1*- and *BRCA2*-mutated BC or OC should also be reexamined. Other cancers (eg, a substantial fraction of *BRCA1/2*–wild type high-grade nonserous OCs) have hallmarks of HRD and might respond to PARP inhibitors. Although it is currently unclear how to best identify PARP inhibitor–responsive cancers, biomarker development trials such as ARIEL2[14] should inform this issue. Patients could then be selected for subsequent trials using promising biomarkers (including FA/HR pathway–mutation testing) rather than cancer type, thereby allowing PARP inhibitors to be tested in various rare cancer subtypes that might never be studied on their own.

Can We Learn More About Drug Resistance in the Clinical Setting?

At present, there is little information about the causes of disease progression after initial clinical response to PARP inhibitors. Optional tumor biopsies on progression that have been incorporated into several PARP inhibitor trials[14,153] should help address this issue. The ability of the off-study biopsies to help guide the next therapy for some patients is an added benefit. Until HRD can be reliably identified through analysis of circulating tumor cells or circulating tumor DNA, we strongly advocate both on- and off-study biopsies in the setting of trials that can productively use them to better understand resistance and ways to circumvent it.

Are Current Expectations Reasonable?

In view of the initial high expectations for PARP inhibitors[90] and disappointment after the negative iniparib phase III trial in BC,[142] it is important to ask what can reasonably be expected of PARP inhibitors. All current models (Fig 1) suggest that these agents kill susceptible cancer cells by perpetuating DNA damage. Thus, their efficacy might be similar to that of other DNA-damaging agents in the same cancers. Accordingly, the similar response rates of olaparib and liposomal doxorubicin in relapsed *BRCA1/2*-mutant OC, albeit with lower toxicity in the olaparib arm,[114] should not be a surprise. Moreover, PARP inhibitors would be expected to select for pre-existing resistant subclones[154,155] just as conventional chemotherapeutic agents do, explaining why the majority of relapsed platinum-responsive OCs progress during PARP inhibitor treatment over the first 18 months.[7] These considerations suggest that PARP inhibitors will benefit suitably chosen patients but will not be curative in advanced disease, even if *BRCA1* or *BRCA2* is mutated. Thus, it will be important to study cancers with prolonged responses to PARP inhibitors[9] to search for even better predictive markers. Moreover, PARP inhibitors will need to be tested in settings of lower disease burden, where their benefit might be even greater (eg, chemoprevention in suitable high-risk groups[156]) as maintenance therapy (Table 1) or in combination with other agents in the advanced-disease setting. Only in this way will the tantalizing activity of these agents be optimized for clinical benefit.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Disclosures provided by the authors are available with this article at www.jco.org.

AUTHOR CONTRIBUTIONS

Conception and design: All authors
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Data analysis and interpretation: All authors
Manuscript writing: All authors
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GLOSSARY TERMS

base excision repair (BER): one of the major DNA repair pathways that repairs simple DNA base lesions, such as the products of deamination, oxidation, and alkylation. In BER, a damaged base is removed by a DNA glycosylase, followed by excision of the resulting sugar phosphate. The small gap left in the DNA helix is then filled in by the sequential action of DNA polymerase and DNA ligase.

***BRCA1*:** a tumor suppressor gene known to play a role in repairing DNA breaks. Mutations in this gene are associated with increased risks of developing breast or ovarian cancer.

***BRCA2*:** a tumor suppressor gene whose protein product is involved in repairing chromosomal damage. Although structurally different from *BRCA1*, *BRCA2* has cellular functions similar to *BRCA1*. *BRCA2* binds to RAD51 to fix DNA breaks caused by irradiation and other environmental agents. Also known as the breast cancer 2 early onset gene.

homologous recombination: genetic recombination whereby nucleotide sequences are exchanged between two similar or identical strands of DNA to facilitate accurate repair of DNA double-strand breaks.

promoter hypermethylation: methylation of the promoter region of a gene, which can lead to DNA silencing as a consequence of the inability of activating transcriptional factors to bind to the promoter region, a process important in gene transcription. In addition, repressor complexes may be attracted to sites of promoter methylation, leading to the formation of inactive chromatin structures.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Poly (ADP-Ribose) Polymerase Inhibitors: Recent Advances and Future Development

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Clare L. Scott
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